STUDIES ON ARTHROPOD CUTICLE. VIII. THE ANTENNAL CUTICLE OF HONEYBEES, WITH PARTICULAR REFERENCE TO THE SENSE PLATES [1, 2]

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The present study was initiated to investigate the question of whether or not the cuticle over chemoreceptors differs other than in thickness from adjacent areas of the cuticle. Due to the mixed distribution of various types of sensilla there is no case where identification of a particular type of sensillum as a chemoreceptor has been absolutely proven. Of the several cases where strong experimental evidence supports the assigned function of chemoreception (Dethier and Chadwick, 1948), the sense plates of honeybees were chosen for study largely because preliminary investigation showed they were more readily prepared for electron microscope examination than other sensilla. Examinations made about ten years ago showed that probably little useful information would be obtained by electron microscope examination of untreated cuticles overlying chemoreceptors; what was needed was an accessible type that could be readily prepared in order to develop extraction and staining analyses for the electron microscope to parallel studies made with the light microscope. Since a single tangential section of a bee's antenna may show several dozen surface views of sense plates satisfactory for electron micrography, these are favorable if not indeed ideal material for such a study.

The answer obtained to the initial question stated above is that the cuticle over these sense plates is different from the cuticle over other parts of the antenna in more ways than just in thickness. Also, the cuticle differs on different types of sensilla. Little information is available on the nature and significance of these qualitative differences but the relatively low lipid content of the epicuticle and the incomplete sclerotization imply relatively ready penetration. Incidentally, an unexpected and novel sequence of sclerotization changes was encountered.

Methods

For this study, honeybees were mostly collected from hives maintained here. The majority of the work was with the worker caste but, at least for electron micro-

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graphs of sense plates, the fully hardened adult drone is similar. Pupal development requires about nine days, and the adult bee emerges from the pupa within a sealed cell and does not chew its way out of this cell until about a day after emergence. For purposes of the present paper, a "teneral adult" is defined as an adult which has been removed from its pupal cell prior to chewing its way out, and an "older adult" is defined as an adult active outside the hive.

Pupae were seriated in terms of developmental stage without any record being kept of the actual hours of development. Ample material being available, sealed pupal cells were opened at random and preliminary seriation made on basis of the eye color, younger stages being more finely seriated by examining the head capsule under a dissecting microscope. Excessive numbers were taken in the desired ranges and finally seriated after sectioning.

Examination of preparations was made with a Leitz microscope equipped with apochromatic lenses, a Leitz research polarized light microscope, or an RCA electron microscope, model EMU. Some preparations were also examined by phase-contrast microscopy. A considerable variety of techniques was employed. Sections were made free hand from fresh material or with a microtome after embedding either *in vacuo* following freeze-drying or *via* benzene following fixation (usually in Carnoy's or Bensley's fluid but sometimes in Bouin's fluid). For the ordinary light microscope, sections were examined unstained, or after extraction with hot water (100° C.) or 10% NaOH solution, or after staining with Mallory's triple stain, Heidenhain's iron haematoxylin, the argentaffin reaction, the Schiff polysaccharide test, or 2% osmic acid. For the polarized light studies, sections and whole mounts were examined in water both as normals and after treatment with 10% NaOH at 100° C. to purify the chitin, or 60% KOH at 160° C. to convert the chitin to chitosan.

For the electron microscope, free-hand tangential sections were prepared, the cells lysed by brief soaking in distilled water, the inner surface then brushed to wipe off the disintegrating cells, the sections rinsed in distilled water, and then, with or without further treatment, placed on bare electron microscope grids and air-dried. This method of preparation limits the EM examination to a single view—that normal to the plane of the surface. Treatments included extraction procedures similar to those used previously in cuticle studies (Richards and Korda, 1948) and an extensive series of reagents treated in the next section. It was noted that in general, less disruption of extremely thin membranes and ultrafine structures followed purification to chitin when Diaphanol [3] was used than when hot alkaline solutions were employed.

Electron Microscope Staining of Cuticle

Objects are resolved in an electron microscope because of differences in density between the object and its surrounding medium. A common limitation in biological work is that inadequate density differences between adjacent parts result in inadequate contrast in the pictures obtained. In general, this limitation may be overcome by increasing the purification of the material, by shadow-casting, or by inducing the deposition of heavy elements in the material. Purification is of limited value when one is studying the normal structure of a heterogeneous system, and shadow-casting is limited to clarifying surface irregularities (best with one type

[3] A saturated solution of chlorine dioxide in 50% acetic acid.

TABLE I

Electron microscope staining reactions with various cuticle samples

Aqueous sols.	% conc.	Chitin *Periplaneta* trachea	Peritrophic membrane *Phormia*	Larval cuticle *Aedes*	Trachea *Periplaneta* adult	Trachea *Phormia* adult	Trachea *Calandra* adult	Crop lining *Phormia*	Crop lining *Periplaneta*	Wing membrane *Aedes*	Wing scales *Aedes*	Tracheal airsac honeybee	Sense plates honeybee
$FeCl_3$	2	—	sl. Gr.	—	?	?	—	?	?	Gr.	—	sl. Gr.	—
$ZrO(NO_3)_2$	0.1–5	—	v. Gr.	v. Gr.	Gr.	sl. D.	—	sl. Gr.	sl. D.	sl. Gr.	—	sl. Gr.	sl. Destr.
$AgNO_3+NH_4OH$	*ca.* 5	—	?	—	var. Sp.	v. l. Sp.	—	—	?	v. sp.	v. l. Sp.	—	f. Sp.
$H_3PW_{12}O_{40}$	2+6	Destr.	?	?	v. Retic.	sl. D.	?	Gr.	?	—	—	var. Gr.	Gr.
OsO_4	2	—	—	sl. D.	v. D.	sl. D.	?	?	sl. D.	v. D.	v. D.	v. D.	sl. D.
$IrCl_4$	1	—	v. Gr.	v. Gr.	sl. Destr.	sl. Gr.	sl. Gr.	sl. D.	sl. Destr.	?	?	v. Gr.	—?
K_2PtCl_6	2	—	sl. Destr.	—	—	—	—	?	?	—	—	sl. Gr.	—
$HgCl_2$	Sat.	—	?	Var.?	—	—	—	—	?	—	—	sl. Gr.	—
Millon reagent	—	—	—	—	—	—	—	—	sl. Destr.	—	—	—	?
$Pb(C_2H_3O_2)_2$	2	—	sl. D.	—	D.	—	?	—	?	—	—	—	—
$Th(NO_3)_4$	2	—	sl. D.	Gr.	v. Retic.	sl. D.	sl. D.	sl. D.	sl. D.	sl. Gr.	—	Gr.	?
$UO_2(NO_3)_2$	2	—	?	?	—	—	—	sl. D.	?	—	—	var. Gr.	—
$NaZn(UO_2)_3$ $(C_2H_3O_2)_9$	2	—	sl. D.	sl. Gr.	v. Retic.	?	sl. Gr.	sl. Gr.	?	sl. Gr.	?	Gr.	—

— = negative; ? = negative or questionable slight effect; D. = distinct darkening, more or less general; Gr. = granular appearance to membrane; Sp. = distinct separated spots; Retic. = spots connected by streaks giving reticulation; Destr. = some structural destructive changes.

v. = very; sl. = slight; f. = fine or minute; l. = large; var. = variable or inconsistent.

of surface discontinuity on an otherwise plane surface; shadow-cast specimens were examined but did not facilitate study of these sense plates). Accordingly, a considerable amount of effort was expended in an attempt to develop a useful set of "stains" which would augment contrast *within* a specimen of cuticle and perhaps even be sufficiently specific in action to aid in cytochemical identifications (Richards, 1952a). The results were discouraging but not nil.

Some of the more interesting data are presented in Table I. These results were obtained after cleaning cuticles in distilled water and then soaking in the reagents at the indicated concentration (usually two days), excess of reagent being removed by washing in water prior to drying on an electron microscope grid. All tests were both replicated and repeated. Inconstancy in thickness of preparations, even in only slightly separated parts of the same membrane, makes it impossible to distinguish between truly negative effects and a possible uniform deposit by comparing control and treated membranes. But structural variability is much more serious than variations in thickness; recordable differences are limited to relatively gross qualitative differences (see Richards and Korda, 1948).

Since no two of the membranes selected as test objects gave identical results, the data emphasize that a considerable amount of interspecific variation is to be expected in the details of cuticle chemistry. The antennal sense plates are not identical in reactions to any of the other membranes tested, but they appear in these tests to be more similar to the cuticle samples from *Phormia* than to the tracheal airsac walls or, as will be brought out in later sections, other cuticularized areas of the same species.

The osmic acid effect (Fig. 23) is negative after extraction of the membrane with hot chloroform. At least for the honeybee airsac it is also negative after exposure to intense electron bombardment (see Hillier *et al.*, 1950). Occasional breaks in the specimens show that the effect is localized in the epicuticle but no separable osmium-positive layer was recognized. Broken preparations of bee airsacs show two principal and separable layers; both the osmic acid and the iridium tetrachloride effects are limited to the outer of these layers but the outer layer itself is still present after refluxing with chloroform and will still react with the iridium chloride. In fact, series of tests with cockroach tracheae, bee airsacs, bee sense plates and mosquito wings and scales showed that extraction with hot chloroform interfered only with the osmic acid reaction. Presumably the osmium is reduced by only the lipids in the cuticles used for these EM tests, and the other compounds react with non-lipid components.

The ammoniacal silver nitrate effect is the standard argentaffin reaction positive for polyamines, polyphenols and certain aldehydes. In cuticle work, a positive reaction is presumed to be due to the presence of a polyphenol substrate for sclerotization (Richards, 1951). The degree of localization of the polyphenol by means of this test is not known but the spots of silver (Fig. 20) are clearly discrete in contrast to the diffuse deposition of osmium (Fig. 23).

Except for the osmic acid and ammoniacal silver nitrate reactions, no explanation can be given for the staining of cuticle by compounds listed in Table I and illustrated in Figures 20–23. Obviously, however, the reactions are not simple ion effects, for the nitrate of thorium is excellent but the chloride is not, and of the three uranyl compounds tested, only the complex one gave good pictures. Several routine histological reagents (chloroplatinic acid, mercuric chloride) were either

negative or so light and general that the effect was not visible. Aqueous solutions of all of the compounds in Table I are acidic except for the ammoniacal silver nitrate which is close to neutrality. Phosphotungstic acid is a general protein precipitant that deposits in at least most of the cuticles used but the alkaline solutions of sodium and potassium tungstate are ineffective; however, many of the ineffective compounds form acidic aqueous solutions and may even be known protein precipitants.

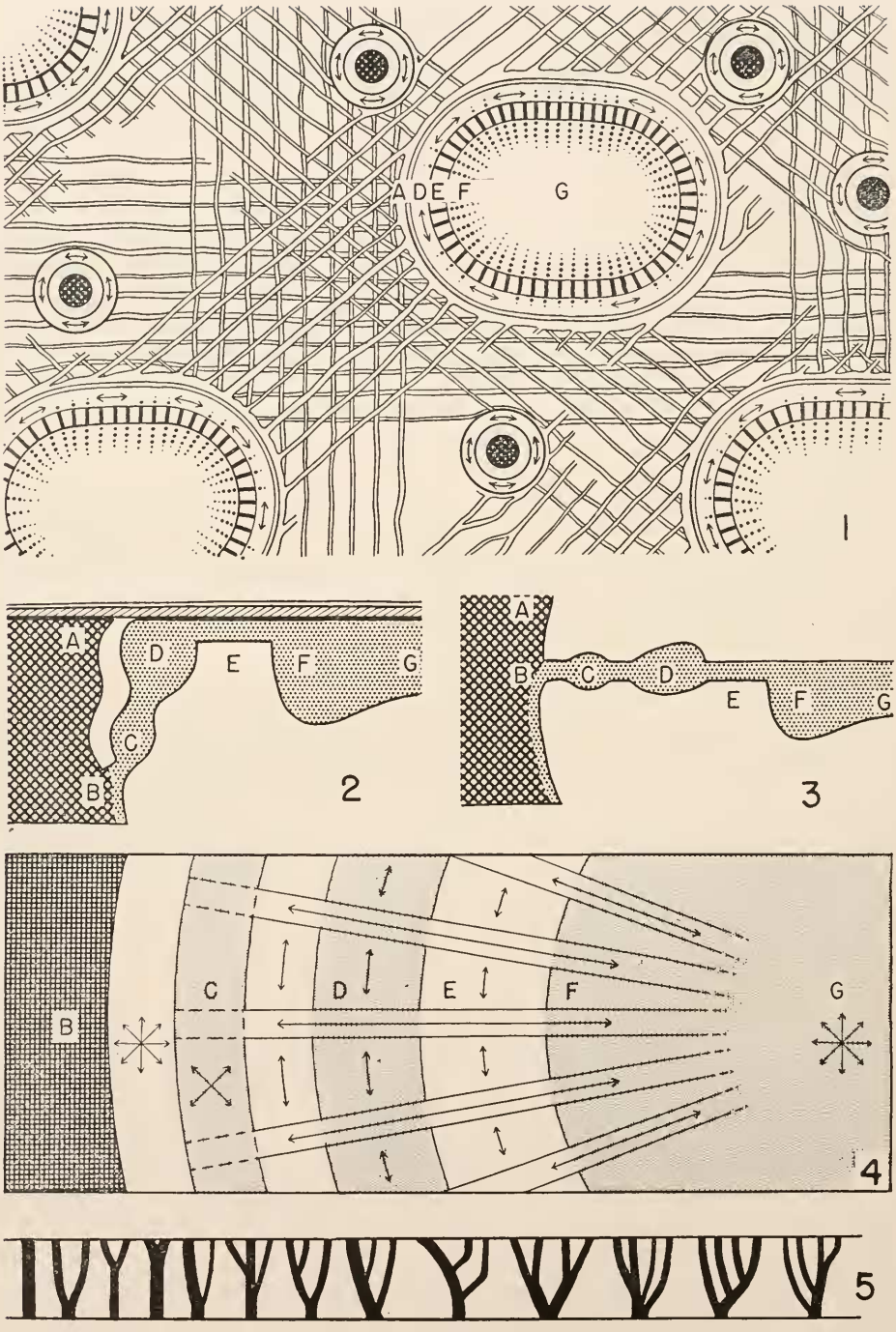
No stain for chitin was found. Since the honeybee airsac is negative to the classic tests for chitin (Richards, 1951), the EM stains do not suggest the possibility of a stain which will react with chitin in cuticle but not with chitin after purification. Chitin and chitosan are both visibly colored by aqueous solutions of iodine plus potassium iodide, but in the electron microscope this treatment results only in a slight overall increase in density which does not seem helpful. It is interesting to note that the chitin chains may not only be de-acetylated to chitosan but also oxidized to the polyaldehyde with periodic acid (involving removal of entire acetylamine side groups) without the micelles appearing different in electron micrographs.

Chitosan can be readily stained by the use of zirconium or titanium tetrachloride in anhydrous organic solvents.[2] In some cases, treatment with these can be used to augment the clarity of micelles (compare Figs. 27 and 29) but the pretreatment required to produce chitosan is violent and leaves little general use for the reagents. Applied to normal cockroach tracheae, these compounds had a somewhat destructive effect or caused dissolution of the endocuticle, depending on the solvent employed; they seemed to give no staining effect to normal cuticle.

Other compounds which have been tested as aqueous solutions (usually at 2%) and found not promising as EM stains for these test cuticles include: NaCl, KCl, $CaCl_2$, $CrCl_2$, $MnCl_2$, $MnSO_4$, $KMnO_4$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, $FeSO_4$, $CoCl_2$, $Na_3Co(NO_2)_6$, $NiCl_2$, $CuSO_4$, $(NH_4)_6Mo_7O_{24}$, $AgNO_3$, $CdCl_2$, $SnCl_4$, $KSbOC_4H_4O_6$, K_7TeO_3, KI, H_5IO_6, KIO_4, CsCl, Cs_2SO_4, $BaCl_2$, Na_2WO_4, K_2WO_4, $KAu(CN)_2$, $HgBr_2$, HgCl, $TlNO_3$, TlI, $Pb(NO_3)_2$, $NaBiO_3$, $BiC_6H_5O_7$, $ThCl_4$, and $UO_2(C_2H_3O_2)_2$. $SbCl_3$ was tested as a saturated solution in chloroform. Several of these compounds are to a greater or less extent destructive to cuticular membranes ($KMnO_4$, $SbCl_3$, $KAu(CN)_6$, etc.).

General Description of the Antennae

The antennae and antennal sense organs of honeybees have been described by a number of authors (see Snodgrass, 1925). The antennae of the worker caste consist of twelve segments with freely movable membranous joints between each segment. The sense plates or sensilla placodea are found only on the distal eight segments, but on these segments are rather densely packed in a band extending from near the basal rim to near the apical rim and circumferentially approximately two-thirds of the distance around the segment. Bands of sense plates are similarly located on each of segments 5–12. In effect, then, there is a band of sense plates extending the length of these combined segments except for interruption at the joints, and a corresponding band free of sense plates on the outer surface. Setae called "sense hairs" are found both scattered around between sense plates and on the band that is free from sense plates. Other types of sensilla are more local in



FIGURES 1–5

distribution. It has been estimated that the antenna of the worker caste has 5000–6000, those of drones about 30,000 sense plates, and that the total number of antennal sense cells is of the order of 500,000 (Snodgrass, 1925).

We can use the above facts, plus data to be presented, as basis for recognizing six types (or areal types) of cuticle on the antenna: (1) sclerotized cuticle between sense plates, (2) sclerotized cuticle in the band devoid of sense plates, (3) sclerotized cuticle devoid of sense plates and setae and forming the basal and apical rims of each segment, (4) soft or unsclerotized cuticle on the intersegmental joints, (5) sclerotized cuticle of sense hairs and sense pegs, and (6) less sclerotized cuticle on sense plates and "sunken setae." Any of these may be compared with cuticle from other parts of the body—such comparison showing that the unorthodox sclerotization of the antennae is not found in the general cuticle of thorax and abdomen and hence is peculiar to the antennae as such, rather than to the honeybee as a species.

Actual presentation of the data, however, will be simpler or at least less redundant if we treat (1) the chitin chain or micelle orientations in the general antennal cuticle, (2) orientations in setae, (3) general structure and orientations in sense plates, (4) development of the epicuticle, (5) development of the procuticle, (6) comparison of the cuticle on various types of sensilla, and (7) comparison to thoracic and abdominal cuticle.

Micelle Orientations in the General Cuticle

Like arthropod cuticle in general (Richards, 1951), the antennal cuticle is laminated. But the laminae in the bee antennae are thin (ca. 0.8 μ) and so low in contrast that they cannot be readily seen in routine observation except at the antennal joints where the cuticle shows a light staining with fuchsin between the thickened laminae (Fig. 31). Unstained sections examined by dark phase-contrast microscopy reveal a general laminate structure. Laminations have been discerned in some sections of frozen-dried preparations stained with haematoxylin. Laminations may also be inferred from alternating lighter and darker bands of birefringence in sections treated with hot water or alkali (Fig. 8), from the presence of crossed Balken layers (see below), and from rows of spots along pore canals of antennae treated with ammoniacal silver nitrate (Fig. 12). The fully developed cuticle is approximately 15 μ thick; accordingly there are some 15–20 laminae.

In the normal, fully developed cuticle no macrofibers or Balken can be seen

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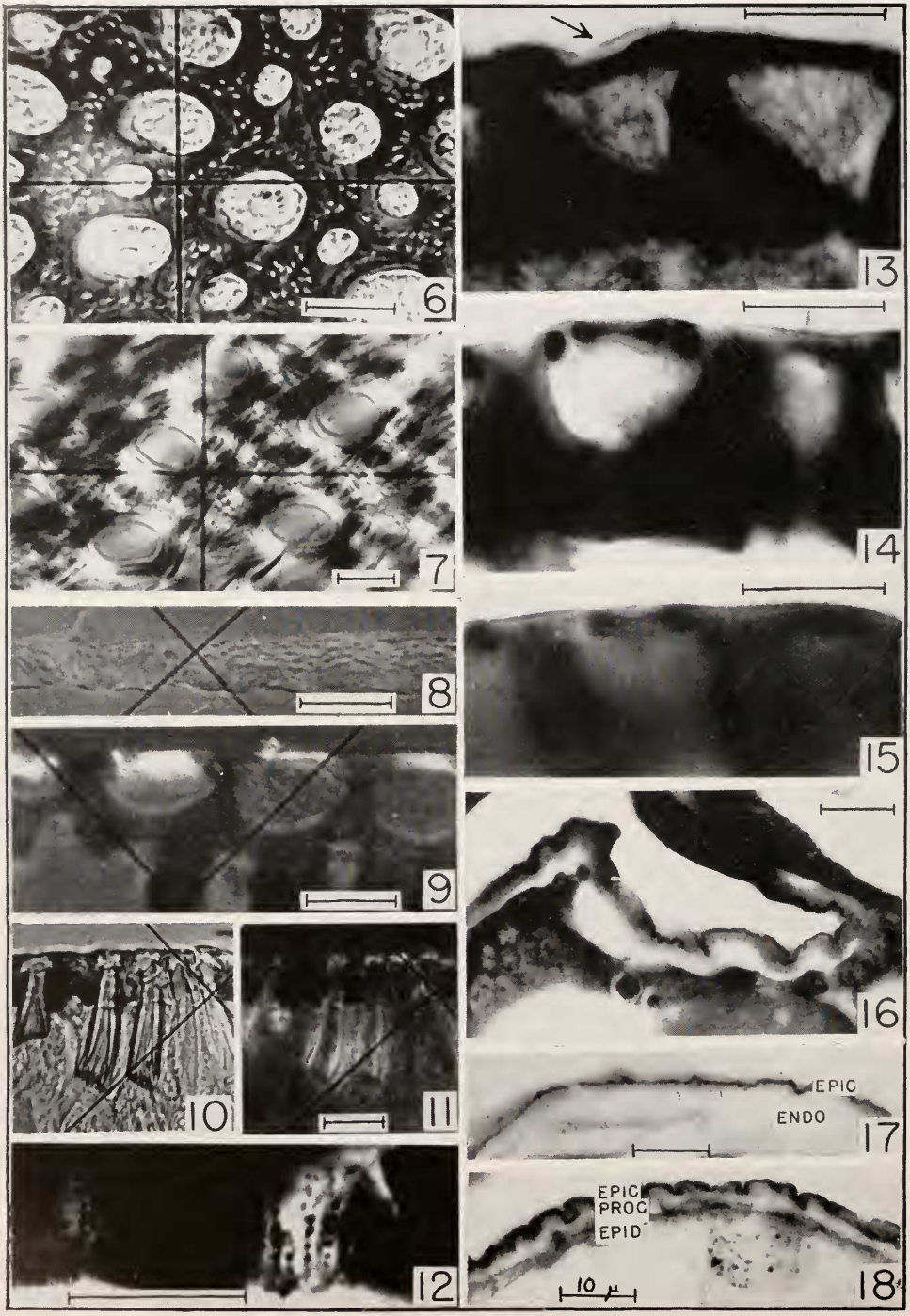
Figure 1. Semi-diagrammatic sketch of a small area of an antennal segment showing distribution of sense plates and sense hairs (sockets as cross-hatched circles), orientations of Balken as seen in specimens purified for chitin, and orientations of micelles in sockets as indicated by arrows. Lettering indicates corresponding parts in Figures 1–4.

Figure 2. Diagrammatic section of normal relations of the cuticle of half a sense plate, its rim or socket, and the adjacent cuticle.

Figure 3. Similar diagrammatic section showing opening out of structure, concomitant with drying, after rupture or removal of the epicuticle. Needed for interpretation of some of the electron microscope pictures (Figs. 19 and 25).

Figure 4. Map of micelle or chitin chain orientations in the cuticle of a sense plate and its socket.

Figure 5. Types of ribs or Balken seen in electron micrographs of the thin membrane between sense plates and the socket rim (see Figs. 19 and 22).



FIGURES 6–18

clearly (Fig. 6), and the cuticle seems nearly isotropic (Fig. 9) (except for setae and sockets, see next section). If the cuticle is treated with water at 100° C. for several days or, better, the chitin purified or further changed to chitosan, a rather coarsely fibrous structure is revealed. These fibers, commonly called Balken, average about 0.5 μ in diameter (range 0.3–1.0 μ), have a more variable spacing which, however, roughly approximates the fiber diameter, and are strongly birefringent with sharp extinctions (Fig. 7). As shown in Figure 1, where for clarity of the diagram the fiber diameters are reduced and the spacings between fibers exaggerated, these fibers occur in layers (Balkenlagen) which apparently correspond to laminae of the cuticle. Four orientations can be recognized forming a crossed-fiber type of orientation: one is longitudinal, one transverse, and two oblique at roughly a 40° angle with the longitudinal set. It was not found possible to count the number of superimposed Balken but since sections are similarly birefringent from outer to inner surface (Fig. 8) the number may well be the same as the total number of laminae. Although four orientations could be readily recognized, the sequence of these was not determined because of technical diffi-

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Magnification of individual photographs indicated by a 10 μ line on each picture.

FIGURE 6. Free-hand tangential section of an antenna fixed in Bensley's fluid and examined unstained in water. Shows distribution of sense plates (large ovals), sense hairs (smaller circles) and pore canals (very small spots).

FIGURE 7. Free-hand tangential section of an antennal cuticle purified to chitosan with hot conc. KOH. Photographed between crossed Nicol prisms with a rotating compensator set to accentuate birefringence along one diagonal while reducing it along the other. Two crossed sets of diagonal Balken are shown. Compare with Figures 1 and 6.

FIGURE 8. Longitudinal section of an antennal cuticle purified for chitin in a 10% NaOH solution at 100° C. Photographed between crossed Nicol prisms.

FIGURE 9. Longitudinal section of an antenna fixed in Bensley's fluid and examined unstained in water. Photographed between crossed Nicol prisms.

FIGURE 10. Long and short sunken setae in a longitudinal section of an antenna fixed in Bensley's fluid and examined unstained in water.

FIGURE 11. Same photographed between crossed Nicol prisms showing the strong, sharply extinguishable, birefringence of socket walls and shaft.

FIGURE 12. Longitudinal section of an antenna that had been abraded with emery powder and then soaked in ammoniacal silver nitrate solution. The thinner parts show precipitation of silver in the pore canals between cuticular laminae.

FIGURE 13. Longitudinal section of an antenna which had been frozen at − 195° C., dried by sublimation at − 35° C., and then embedded in paraffin *in vacuo*. Stained with Mallory's triple stain. Arrow shows area where the epicuticle is separated from the surface of a sense plate.

FIGURE 14. Longitudinal section of an antenna fixed in cold Bouin's fluid and stained with Heidenhain's haematoxylin. Shows differentiation of the sense plate socket and of the epicuticle.

FIGURE 15. Another photograph from the same section but from the region where sense hairs but no sense plates are found.

FIGURE 16. Section of a pupal antenna stained with Mallory's triple stain and photographed with a green filter. Shows the future membrane between antennal segments, and developing sclerotization at each end of this membrane.

FIGURE 17. Section of the membrane between antennal segments of a teneral adult stained with the periodic acid-Schiff reagent method for polysaccharides. Epicuticle intense red; endocuticle faint pink.

FIGURE 18. Similar to preceding but from a late pupa. Epicuticle intense red; procuticle and epidermal cells light pink.

culties arising from the fact that after alkali treatment one set of fibers droops into the interstices of the next. It can be recorded that in all of the several dozen specimens examined, the outermost layers of fibers were oblique.

At least the outermost sets of Balken fuse into a rim around the sockets of setae and other sensilla. Presumably lower layers do likewise. Here and there a Balken fiber can clearly be seen to branch but without any constant angle being involved; some gradually separate at a small angle, others branch off almost at a right angle and then turn abruptly to become parallel.

There seem to be two general possibilities for the origin of Balken fibers: either they are giant micelles or they are micellar aggregates (Richards, 1951). Branched Balken of diverse diameters, illustrated by Langner (1937) for diplopod cuticles, favor the idea of micellar aggregates (also, Biedermann (1903) reported that Balken seem to be composed of more minute fibers). This is strongly supported for the Balken of honeybee antennae by the fact that electron micrographs of the frayed edges of antennal sections (Fig. 28) sometimes show Balken separated into smaller fibrils that extend down to micellar dimensions (Richards and Korda, 1948). Further support for the idea that Balken are micellar aggregates comes from the purely chemical separation of "submicroscopic Balken" of sense plates into fibrils of micellar dimensions (Figs. 27, 29). These data are consistent with Kühnelt's (1928) suggestion that Balkenlagen are only specialized cuticular laminae. No evidence was obtained as to why successive laminae show different orientations and so produce the crossed-fiber type of structure.

The Balken sets extend around the antennal segment and appear entirely similar in the bands with and without sense plates. However, at the ends of each antennal segment the Balken lose their identity in the basal and apical rings. These areas appear to lack a Balken type structure, or, if one prefers, to be a single larger Balken (since they are an indivisible band of tightly packed, paralleled molecular chains). These apical rings show sharp extinction with the chitin micelles extending circumferentially (= tangentially) around the antennal segment.

The author was somewhat surprised to find the optical effects showing that the soft intersegmental membranes of the antennae have closely paralleled micelles. In this case there seems to be no tendency for the micelles to aggregate into Balken —at least the membrane appears uniformly birefringent. The sharp extinction shows that this membrane does not have a crossed-fiber type of structure; the micelles are parallel to the axis of the antenna and hence at a 90° angle to those they join at the apical and basal ends of each segment.

Longitudinal sections show a laminate structure between crossed Nicols due to different amplitudes of birefringence in successive laminae (Fig. 8). A similar picture from tick cuticle has been published by Schulze (1932). Such an effect is to be expected from any single plane through sets of crossed fibers even though each lamina would be equally birefringent if properly oriented.

The size and spacing of the relatively large pore canals (Fig. 6) is consistent with their passing between Balken and through the interstices of crossed Balken sets.

Micelle Orientations in Setae

The cuticle of setae ("sense hairs") and setal sockets, unlike the general surface cuticle, is distinctly birefringent in its normal state (Figs. 9, 11). The explanation

of this difference is not known although several suggestions are possible (see Richards, 1951, p. 84). The amplitude of setal birefringence can be increased by purification of the chitin.

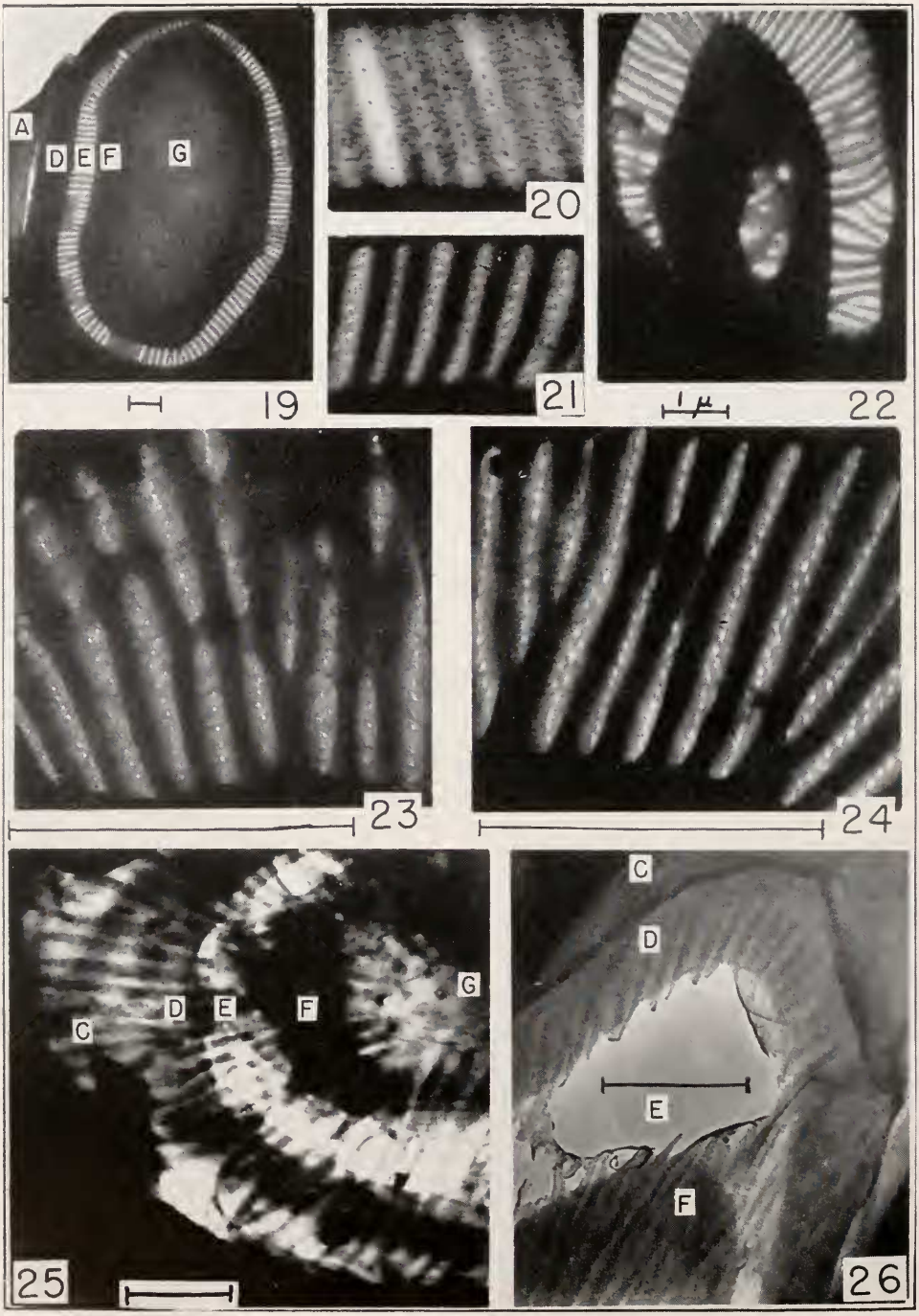
Both the sense hairs and their socket rims show sharp extinction indicating a high degree of orientation. The micelles are parallel to the setal axis along the shaft, radial in the thin soft socket membrane, and circumferential or tangential in the socket rim. The shaft of sense pegs is similar but the sockets show less birefringence (details of which were not determined). Both long and short sunken setae (Figs. 10–11) show orientations similar to those of tactile setae; since the tormogen membrane is, in effect, stretched and elongated to form the setal pocket, the radial micelles of this membrane are also oriented approximately parallel to those of the setal shaft.

Clearly, micelle orientations do not depend on sclerotization for stability because equally sharp extinctions are given by soft membranes (staining blue with Mallory's stain), by incompletely sclerotized membranes (red with Mallory's stain), and by heavily sclerotized membranes (refractory to stains). The extensive analysis of *Drosophila* setae by Lees and Picken (1945) suggests that growth pressures produce the longitudinal orientation in setal walls while they are being laid down.

The General Structure and Micelle Orientations of Sense Plates

The sense plates, or cuticles thereof, are thin complicated membranes overlying a group of cells that project most of the way through the cuticle (Figs. 33, 46). In surface view (Figs. 1, 6, 19) they are seen to be more or less oval structures whose long axes tend to parallel the axis of the antenna. A central plate (Fig. 1, *F–G*) is surrounded by a very thin membrane (*E*) which joins a thick rim (*D*) which in turn is surrounded by a socket rim of the general cuticle (*A*) formed by the fusion of Balken. The sensillum attains this recognizable differentiation very early in cuticle development (Figs. 38–39) but the following notes are based entirely on the condition found in the fully formed organ on adult antennae. Some authors have suggested that these peculiar structures can be derived, in a phylogenetic sense, from setae by assuming that the setal shaft is flattened down to become the central plate (Snodgrass, 1925).

In cross or longitudinal section (Fig. 2), it is seen that a double-layered epicuticle extends continuously across the sense plate (*D–G*) and adjacent cuticle (*A*). Beneath the epicuticle is a continuous chitin-protein cuticle. This is only partially sclerotized in the sense plate and its rim (*C–G*) but heavily sclerotized in the surrounding cuticle (*A–B*). If the epicuticle is removed or disrupted and the sense plate caused to shrink under electron bombardment, the structure straightens out in the manner diagrammed in Figure 3. There is no question that the rim is firmly attached to the cuticle at *B–C* (this can occasionally be seen in stained sections examined with the light microscope too), but it is not certain whether the chitin micelles fuse into those of the rim at this point or extend inwardly to form a lining of the cavity which contains the cells of the sensillum. It is also uncertain whether the space diagrammed between *A* and *C–D* is a real space or only a line of weak linkages; it does, however, coincide with a line of differentiation demonstrable with either Mallory's or Heidenhain's stains (Fig. 14).



FIGURES 19–26

Examination with an electron microscope reveals that the thin membrane is traversed by some 120–150 radially arranged thickenings (Fig. 19). Over 80% of these are simple rods, well over 90% are either simple or bifurcated rods, but a small percentage of triply, quadruply or irregularly branched ones are seen (Fig. 5). Examination of extracted specimens shows that these thickenings are rod-like structures that may be compared to the Balken of the general antennal cuticle from which they appear to differ only in being beyond the limits of resolution by visible light microscopy. These "submicroscopic Balken" extend centrally into the middle of the sense plate (Fig. 1) and then lose their radial orientation (Fig. 22); they also extend through the rim *D* and on at least to *C* maintaining their radial orientation (Figs. 25–26). By prolonged and rather violent chemical treatments these Balken can be broken into microfibers of micellar dimensions (Fig. 29) and "stained" with zirconium or titanium to increase their contrast (Fig. 27).

In addition to the radially arranged micelles aggregated into Balken, there are circumferential (= tangential) micelles at least in areas *D* and *E*. For the rim *D* this is shown by polarized light effects. Figure 7 shows that the rims are birefringent in surface view; Figure 9 shows that the rims are also birefringent along radii of the sense plates but isotropic where the rim is cut in cross section. Accordingly, since the optic axis of chitin micelles is known to parallel the fiber axis (Richards, 1951), the micelles must be circumferential. Electron micrographs not included among the illustrations for the present paper indicate that a single layer of circumferential micelles extends around the membrane *E* and across the radially arranged Balken (specimens treated with iridium tetrachloride). It seems highly probable that the rows of holes seen in the membrane between Balken in Figures 21, 23 and

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Magnification of individual electron micrographs indicated by a 1 μ line on most of the pictures.

FIGURE 19. Relatively low power micrograph showing structure of the cuticle of a sense plate from a preparation cleaned in distilled water at room temperature (= normal). Lettering as in Figures 1–4.

FIGURE 20. Micrograph of a small portion of the thin membrane area (region *E* of Figs. 1–4 and 19) after soaking in ammoniacal silver nitrate solution (2 days). The dark spots represent silver deposits of the order of 100–200 Å. Magnification approximately equal to that of Figure 24.

FIGURE 21. A similar area of thin membrane after soaking in a 5% solution of phosphotungstic acid or $H_3PW_{12}O_{40}$ (1 day). The light spots appear to be breaks in the epicuticle between circumferential micelles (see text). Magnification as for preceding.

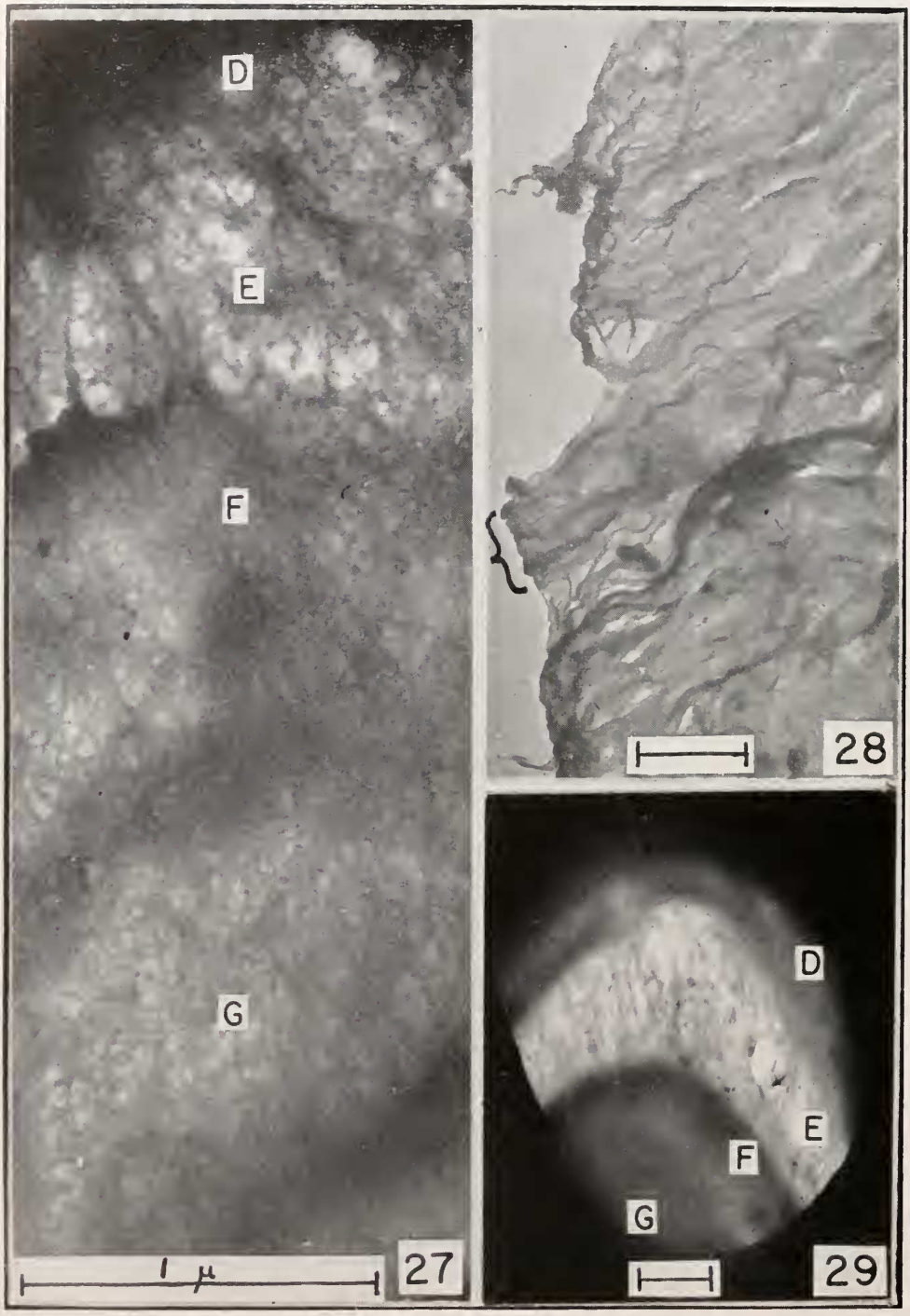
FIGURE 22. Another relatively low power micrograph of the cuticle of a sense plate after extraction in distilled water at 100° C. (1 day). Shows dispersion of a central area of the plate revealing thickenings.

FIGURE 23. A small portion of the thin membrane after soaking in a 2% solution of osmic acid or OsO_4 (1 day). The light spots are similar to those in Figure 21 (see text).

FIGURE 24. A small portion of the thin membrane after soaking in Diaphanol (3 days) following initial cleaning in distilled water and refluxing with chloroform.

FIGURE 25. One end of a sense plate after soaking in 30% H_2O_2 (7 days). The field of view represents the areas from *C* to *G* of Figures 2–4. Note the continuity of the thickenings (Balken) from *C* through *F*.

FIGURE 26. One end of a sense plate after soaking in Diaphanol (8 weeks). Shrinkage and electron destruction is obvious but note the cross striations on and between the radial thickenings.



FIGURES 27–29

24 represent spaces between such circumferential micelles,[4] and that the cross striations between radial thickenings in Figure 26 do likewise.

A composite survey of the data on micelle orientations is given in Figure 4. Recapitulating, evidence for tangential orientation in the thick rim *D* is based on optical effects; radial orientation in *E–F* is based on both optical effects and electron micrographs; all other orientations indicated are based entirely on electron microscope pictures.

Dimensions of the various portions of sense plates are: the overall surface size averages 10 × 14 μ; the central plate averages 7.5 × 11 μ, and is 0.5–1.25 μ thick at the center (*G*) and 1–2 μ thick at the edge (*F*); the thin circumferential membrane normally appears to be about 0.6 μ broad in surface view but displaced sensilla and many electron microscope pictures show it as 1–1.5 μ broad (apparently due to the membrane actually extending part way across the rim *D*). The Balken in this membrane average 0.1 μ in diameter when dry with a range of 0.08–0.15 μ except for a few branched ones where the common trunk is as large as 0.2 μ. The membrane between these Balken (excluding the epicuticle which is several tenths of a micron thick) is only a few hundred Ångstrom units thick when dry; how thick it may be in the living insect is unknown but it can scarcely be more than a few tenths of a micron.

The Epicuticle and Its Development

The epicuticle forms a continuous, slightly birefringent outer sheath over the antenna, but being only a fraction of a micron thick, it is difficult to study. Its continuity without interruption across the sense plate from the surrounding cuticle (Fig. 2) is shown by certain slides, prepared by the freeze-drying technique, in which the epicuticle became separated from the underlying chitin-protein layer at some places (Fig. 13); continuity is also indicated by certain broken preparations which, when examined in the electron microscope, show a thin layer that seems certainly epicuticle projecting without apparent discontinuity from the sense plate, the surrounding cuticle and the minute intervening space. Apparent continuity can also be observed in ordinary stained sections viewed with a light microscope (Fig.

[4] In Figures 21 and 23 the epicuticle has apparently broken under electron bombardment where most strained and least supported, *i.e.*, between the circumferential micelles; in Figure 24 the epicuticle has been removed chemically. These spaces are illustrative of one of the most serious technical difficulties involved in electron microscopy of insect cuticle, namely, that cuticle (and chitin) shrinks a considerable amount under electron bombardment (and may be readily "burned up" or distorted beyond recognition if the electron beam is made intense).

Magnification of individual electron micrographs indicated by a 1 μ line on each picture.

Figure 27. Portion of a sense plate after treatment, successively, in dist. H_2O, 30% H_2O_2 (9 days), 60% KOH at 160° C. (20 min.), and alcoholic 5% $TiCl_4$ (30 min.). Titanium reduced onto exposed amine groups acts as an electron stain to increase the clarity of the micelles. Lettering as in Figures 1–4 and 25–26.

Figure 28. Frayed end of a section of sclerotized cuticle between sense plates after treatment with 10% NaOH at 100° C. (1 day). The bracket indicates a single Balken visible with the light microscope.

Figure 29. One end of a sense plate after soaking in 60% KOH at room temperature (3 days) and then heating to 160° C. (15 min.). Shows separation of the radial thickenings into smaller fibrils of somewhat variable width, and, in comparison with Figure 27, low contrast correlated with the absence of an electron stain.

14) but, as Blower (1951) has emphasized, diffraction effects along the cuticle edge may create serious illusions in work on the epicuticle.

The epicuticle was not actually separated into two layers by any of the methods employed but it appears nonetheless double, at least in the sense that the outer layer or outer surface is stainable with osmic acid and Heidenhain's haematoxylin (Figs. 14–15). In comparison to the epicuticle over the general surface of the antenna, the epicuticle seems to be thicker on the intersegmental membranes (Figs. 16–18) and thinner across the sense plates but the thicknesses are too small for accurate measurement in these sections.

Data on the stainability of the epicuticle of various antennal areas at several developmental stages are summarized in Table II. In very early stages of devel-

TABLE II

Changes in staining reactions of the epicuticle during development as seen with the light microscope. Upper term refers to outer layer (when distinguishable), lower term to inner layer of epicuticle; dash indicates refractory to staining by the indicated method

	Violet eyes, colorless antennae =Figure 36			Purple eyes, yellowish antennae =Figure 39			Teneral adult			Older adult		
	I.M.	S.P.	Scl.	I.M.	S.P.	Scl.	I.M.	S.P.	Scl.	I.M.	S.P.	Scl.
Schiff polysaccharide test	Red	Red	Red	Red Pink	Red Pink	Red Pink	Red Pink	Pink —	— —	Red Pink	— —	— —
Argentaffin test				Spots? —	Spots? —	Spots? —	— —	— —	Gray —	— —	— —	Gray —
Osmic acid 2%				— —	— —	— —	— —	— —	Black —	— —	— —	Black —
Mallory's triple stain	Red	Blue	Blue	Red Red	Red ?	Red —	Red Pink	Purple —	— —	Red Pink	Purplish —	— —
Heidenhain's iron haematoxylin				Brown Black	Brown Black	Brown Black	Gray —	Black —	Gray —	— —	Gray —	Black —

I.M. = intersegmental membrane between antennal segments.
S.P. = sense plates on antennae.
Scl. = sclerotized general surface of antennae.

opment in the pupa it is extremely difficult to differentiate between the epicuticle and a possible precipitate of the molting fluid on the surface (Figs. 35–38). Mallory's stain colors both epicuticle and molting fluid blue on the future sclerotized areas and gives differentiation only at the future intersegmental region where the epicuticle is colored red, but the Schiff polysaccharide test (Fig. 18) fails to stain the precipitated molting fluid. During these early stages there appears to be a definite membrane (blue with Mallory's stain) outside the developing adult cuticle (Figs. 36–38). The nature of this membrane remains to be studied but the fact that developing sense hairs and sense pegs seem to push into it or push it outward suggest that it is not a part of the forming adult cuticle. Perhaps it is the same as an "ecdysial membrane" found by C. M. Williams and V. Passoneau (unpublished) in *Cecropia* pupae—they consider it to be an undigested innermost lamina of the pupal cuticle.

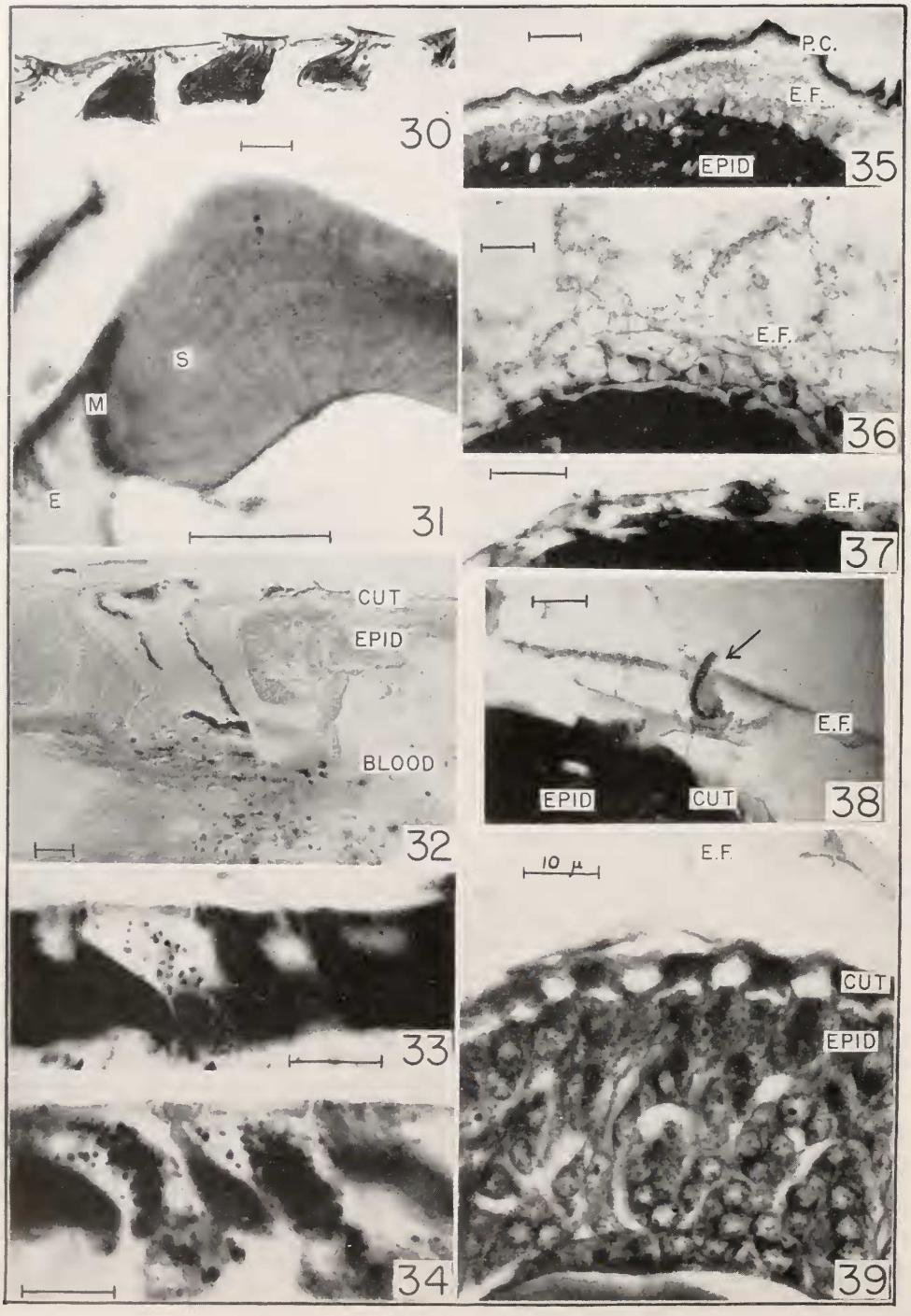
There is even more uncertainty about the interpretation of staining obtained with the argentaffin reaction; clearly the surface precipitate in Figure 32 is considerably thicker than the entire epicuticle, and clearly the silver deposits revealed by electron microscopy are more restricted than the sclerotization effect (Fig. 20 and Richards, 1951, Fig. 64B)—the data make one wonder about the degree of localization obtainable with the argentaffin reaction but we lack detailed knowledge of the chemical reactions involved.

Data presented in Table II show that the epicuticle at first stains uniformly over the antenna. An early differentiation giving different isoelectric points in different areas is implied by the results with Mallory's stain. Subsequently, differentiation results in the ability to recognize at least three types found respectively on the intersegmental membrane, the general sclerite surface, and the sense plates (Richards, 1952b). With Mallory's stain, Heidenhain's haematoxylin and the Schiff polysaccharide test, the change with time is due to partial or complete masking of the reactions given by early stages; with the argentaffin and osmium tests, the change is due to initiation of a positive reaction.

The precipitation of osmium from a 2% solution of osmic acid is detectable with the light microscope only on the general sclerite surface. However, use of the electron microscope reveals a general but very light deposit across the sense plate membranes (Fig. 23). This osmium reaction is prevented when the preparation is previously extracted with hot chloroform. It follows that a very small amount of lipid is to be found in the epicuticle across sense plates. Comparison of osmium-treated preparations with chloroform-extracted ones failed to reveal any recognizable lipid or wax layer, but it should be remembered that the electron microscope studies were limited to surface views where superimposed layers can be distinguished with certainty only when one layer ruptures and peels away from the other.

It has already been mentioned in a previous section that the epicuticle of the thin membrane over sense plates may show rows of minute holes (50–300 Å) after certain treatments (Figs. 21, 23). No such perforations are to be seen in normal preparations with nearly the same resolution. It is thought by the author that these holes in treated epicuticles are produced by the destructive effect of electron bombardment at points of lesser support by underlying circumferential micelles in the chitin-protein layer. Similar holes are to be found in preparations from which the epicuticle has been removed (Fig. 24). There is nothing to indicate that such holes are to be found in normal membranes.

The recognizable subdivisions of the epicuticle have been referred to by the noncommital terms "outer" and "inner." The inner subdivision develops a natural amber color and early becomes resistant to staining; it apparently is the same as the "cuticulin layer" of Wigglesworth (1947, 1948) and the "protein epicuticle" of Dennell (1946). No pore canals (readily stained in the procuticle) could be traced into this layer at any of the stages examined. The outer subdivision is stainable with haematoxylin, osmic acid and ammoniacal silver nitrate; it accordingly is thought to contain protein, lipid and polyphenol but as far as could be determined these occur together, not in separate superimposed layers. The outer subdivision, then, compares with Dennell's "lipid epicuticle" and with Wigglesworth's "polyphenol layer" plus "wax layer." By definition, no tectocuticle (cement layer) can be present since no dermal glands are to be found (also, no layer was separated off the surface by heating in chloroform).



FIGURES 30–39

The Chitin-Protein Cuticle and Its Development

When the adult cuticle begins to develop it is at first a single thin layer that represents the epicuticle (Fig. 36). Within the next few hours there is a rapid increase in thickness, involving the epicuticle becoming visibly double and initiation of production of the chitin-protein procuticle (Richards, 1951). Fortunately for the present study, the sense plates become recognizable very early and can be seen to have already differentiated into central plate, circumferential membrane and rim by the time the cuticle is 1–2 μ thick (Fig. 38). Further development to full thickness (ca. 15 μ) occurs within a day, and events leading to sclerotization commence while the procuticle is still increasing in thickness.

Before treating the unusual details of sclerotization in these antennae, mention should be made of a paper now in press by Schatz (1952) proposing a terminology that will be followed here. Studying sclerotization in various insects, using primarily the differential coloring obtained with Mallory's triple stain (supplemented by haematoxylin and the Millon reaction), Schatz concluded that differentiation of the original soft procuticle is at least descriptively a two-stage process, and that the differentiation results in three rather than two recognizable subdivisions of the chitin-protein portion of the cuticle. The original transparent procuticle stains blue with Mallory's; a portion of this, while remaining transparent, changes to staining red with Mallory's; a portion of this last subsequently becomes refractory to staining and simultaneously develops its own amber or brown color. Classically, the hard colored part is called "exocuticle," the softer, transparent part "endocuticle."

Magnification of individual photographs indicated by a 10 μ line on each picture.

Figure 30. Unstained longitudinal section of the cuticle from a split antenna extracted in dist. H_2O at 100° C. (2.5 days). Shows failure of the treatment to remove the pigmentation.

Figure 31. Longitudinal section through the swollen cuticle at the base of an antennal segment of an old adult (Mallory's triple stain). Shows swollen laminae made distinct by thin fuchsinophile lines, pore canals stained by fuchsin, and dark red band (*M*) separating sclerite (*S*) from the blue-staining membrane (*E*).

Figure 32. Longitudinal section of a pupal antenna that was removed from the pupal case, soaked in ammoniacal silver nitrate (1 hour), then fixed in Carnoy's fluid, sectioned and mounted without additional staining. Note the argentaffin positive granules on the surface of the cuticle and in the blood cavity within the antenna.

Figures 33–34. Longitudinal sections of the antenna of an old adult. The antenna was split longitudinally and then treated with ammoniacal silver nitrate in the same manner as the preceding. Shows dense aggregations of argentaffin-positive granules in the cells of the sense plates, and (less clearly) the absence of such from cells of the sense hairs and general epidermis.

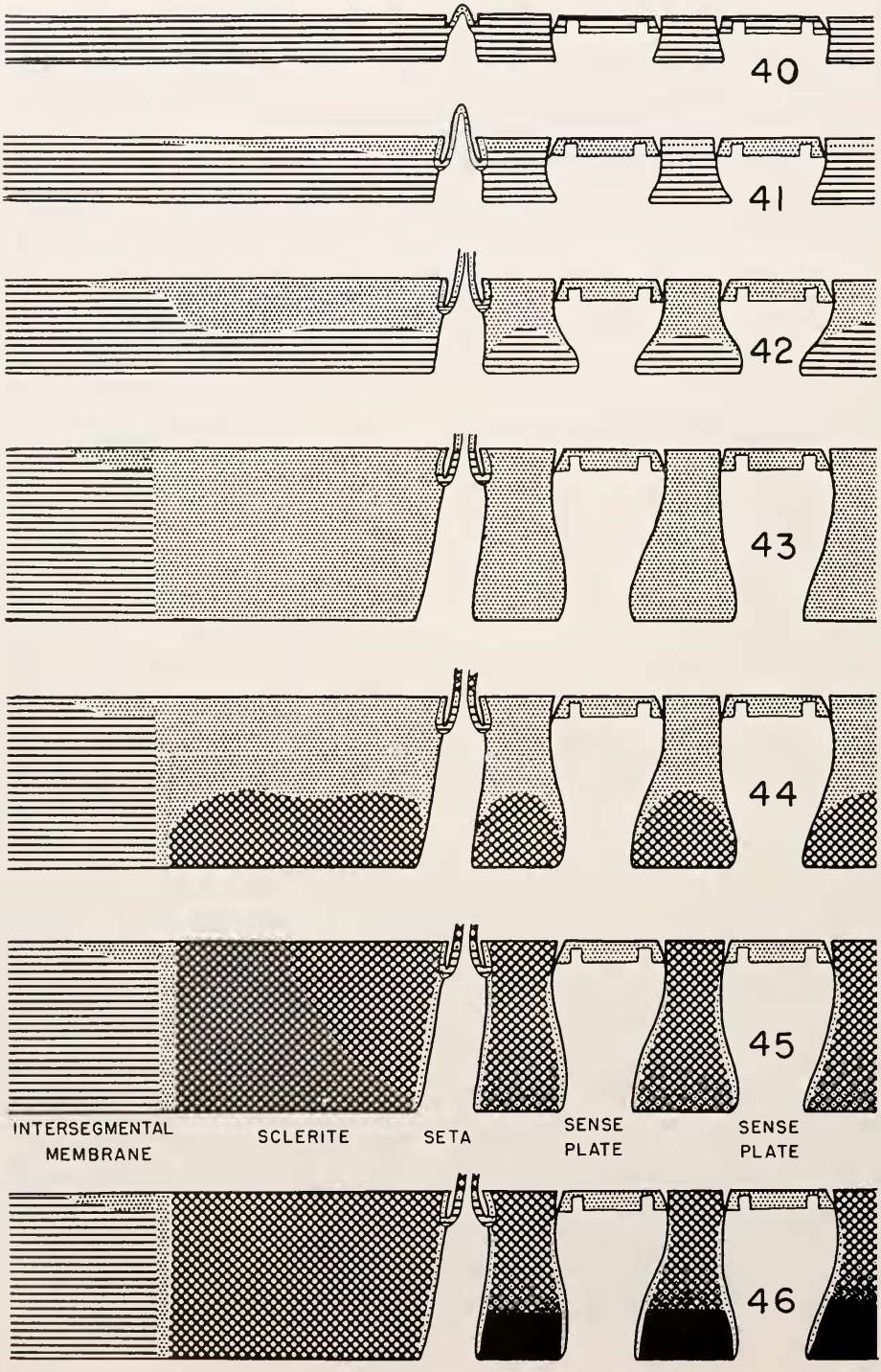
Figure 35. Cross section of the antenna of a pupa fixed in Carnoy's fluid, stained with Mallory's triple stain, and photographed with a green filter. This is an early stage in cuticle development; the print was made dark to show the precipitated molting fluid (stained blue) between the pupal cuticle (*P.C.*) and the epidermal cells (*EPID*).

Figure 36. A similarly prepared section from a slightly older pupa. Note developing sense hairs projecting into precipitating molting fluid (*E.F.*). Corresponds to an early part of the stage diagrammed in Figure 40.

Figure 37. Another similarly prepared section from a pupa of approximately the same age as the preceding. Note the distinct extra membrane.

Figure 38. A similarly prepared section from a slightly older pupa when the adult cuticle is 1–2 μ thick. Note the displaced half of a sense plate (arrow) which is lying in surface view on top of the extra membrane. Corresponds to a late part of the stage diagrammed in Figure 40.

Figure 39. A similarly prepared section from an older pupa when the cuticle (*CUT*) is nearly half the final thickness. Corresponds to Figure 42.



FIGURES 40–46

In a fully hardened cuticle, hard exocuticle is usually separated from blue-staining endocuticle by a red-staining line or band, and hard exocuticle goes through a red-staining stage during its development. Schatz proposes that the hard colored part be called exocuticle, the transparent but fuchsin-staining part be called mesocuticle, and the transparent and still blue-staining part be called endocuticle. Incidentally, the mesocuticle was the only one of these found to stain with Heidenhain's haematoxylin and with the Millon reagent. A somewhat similar picture has recently been presented by Ito (1951) and Blower (1951). Blower has proposed the term "prosclerotin" for the substance giving the Millon reaction. In the present case a purely descriptive histological term seems desirable, and mesocuticle will be used here without implying any particular chemical meaning.

Sclerotization of the bee antennae commences when the cuticle is ¼ to ⅓ its eventual thickness, and follows the novel sequence diagrammed in Figures 40–46. At first the entire procuticle stains blue with Mallory's stain (Fig. 40). Then, beginning at the outer surface, a change commences (Fig. 41) and spreads inwardly (Figs. 16, 42–43) transforming all of the procuticle of future sclerotization areas into red-staining mesocuticle. The epicuticle is not altered in its staining reactions at this time, and still seems blue over future sclerites; setae are slightly in advance of the general cuticle; the procuticle of sense plates has reached its final staining condition by the stage shown in Figure 42 and will not change further; the future intersegmental membrane thickens without changing its staining reactions; and by the time this change of future sclerite areas to mesocuticle is complete, the cuticle has reached its full thickness. Then, and this is the novelty involved, *beginning at the inner surface and spreading outwardly* (Fig. 44), the mesocuticle becomes amber colored and refractory to staining in prospective sclerite areas, *i.e.*, becomes typical exocuticle throughout its entire thickness. Subsequent to completion of this sclerotization (Fig. 45) the inner ⅓ to ½ becomes increasingly brown and eventually quite dark (Fig. 46), but for some unknown reason this additional browning of the inner portion of the exocuticle occurs only in the exocuticle between sense plates—the exocuticle of the band free from sense plates remains unmodified.

Summarizing for the condition found in the chitin-protein portion of the cuticle of the fully developed adult (Fig. 46): The intersegmental membrane stains blue and is accordingly all endocuticle except near the sclerites where the outer portion is red-staining mesocuticle. The intersegmental membrane is separated from sclerite areas by a red line of mesocuticle (Fig. 31). The sclerotized areas are completely exocuticle except for thin red layers lining the setal and sense plate apertures, but the inner portion of areas between sense plates is modified by additional darkening. Sense hairs and sense pegs become all exocuticle except for the tormogen membrane, whereas sunken setae and sense plates remain in the mesocuticle stage and have a similarly staining tormogen membrane.

This sequence in sclerotization is readily rationalized if we assume a two-stage

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FIGURES 40–46. Diagrammatic presentation of the sequence of developmental stages of the procuticle of honeybee antennae as shown by preparations stained with Mallory's triple stain. Parallel lines = procuticle and, in later stages, endocuticle; stipple = mesocuticle; cross-hatch = exocuticle; solid black = exocuticle modified by additional dark pigment. See text for explanation.

process controlled by two agents (enzymes?), one of which is effectively located at the outer surface, the other at or beyond the inner surface. Assuming two such agents, there is no known reason why they might not have the reverse distribution to that recorded for these antennae (and this is indeed what Schatz (1952) records for the cockroach *Blatta orientalis*), or both be active from the outer surface (as seems to be generally true), or both be active from the inner surface (not yet recorded).

The distribution of granules reacting with ammoniacal silver nitrate has one interesting feature. In the earliest stages, the entire antenna is negative to the argentaffin test. Somewhat later, numerous positive granules are seen in the blood space and on the outer surface of the developing cuticle (Fig. 32). Still later, positive granules are found in the epidermal cells over the entire antenna. Then, as sclerotization is completed, these granules disappear from the epidermal cells except along the soft intersegmental membrane and in the cluster of cells under sense plates. In the adult, the cells under sense plates are loaded to various degrees with large argentophile granules (Figs. 33–34) whereas cells under the general sclerite surface and under sense hairs and sense pegs are free of such granules. The argentaffin reaction is only semispecific and correlation is not proof, but the data are consistent with the idea that such granules supply the polyphenol derivative used as substrate to change the mesocuticle to exocuticle, and that this change is controlled not by which cells are permeable to this substrate from the blood but by which cells pass it out into the cuticle. A cursory check of argentaffin reactions in developing cuticles of *Blatta, Oncopeltus, Phormia* and *Apis* thoraces suggests that this argentaffin sequence may be another peculiarity of honeybee antennae.

The diffuse dark color developed in the inner part of the exocuticle between sense plates is no staining effect. It can be seen in free-hand sections examined in water and in unstained frozen-dried preparations. It is only slightly if at all decreased in intensity by prolonged treatment with hot water (Fig. 30), a treatment which changes the cuticle from virtually isotropic to moderately birefringent. It is not visibly affected by Carnoy's fixing fluid or common solvents such as alcohol, acetone, chloroform, etc. Conceivably it might be a melanin formed from excess substrate not needed for sclerotization.

Incidentally, the fully hardened exocuticle of adult antennae is darkened or blackened by ammoniacal silver nitrate and 2% osmic acid. The darkening by OsO_4 is not prevented by previous refluxing with boiling chloroform and so must be due to some agent not removed by chloroform or destroyed by heating to 61° C.

The relatively large pore canals (Fig. 6) follow a straight or slightly wavy course from the epidermis to the epicuticle, penetrating between interstices of the crossed Balken. The pore canals are stained by ammoniacal silver nitrate, Heidenhain's haematoxylin and Mallory's stain (red); they are not stained by osmic acid or by the Schiff polysaccharide test. Even in the old adult, sections in which the epidermal cells are slightly separated from the cuticle show cytoplasmic strands extending to and seemingly into the pore canals, and electron micrographs of tangential sections which have been soaked in distilled water show the canals as holes. It follows that these canals never become plugged with cuticle. The only notable point is the curious observation that in regions thin enough to see through in sections stained with ammoniacal silver nitrate, rows of spots occur along the pore

canals at intervals agreeing with the spacing of cuticular laminae. Similar precipitation of silver spots along pore canals of *Tenebrio* cuticles was obtained by Day (1949) using a technique thought by some to be specific for ascorbic acid.

Comparison of Various Sensillum Types

The sense hairs and sense pegs differ in cuticle thickness but the cuticles of both are fully sclerotized in the adult and appear completely negative to staining by Mallory's and Heidenhain's stains, 2% osmic acid and the Schiff polysaccharide test. The tormogen membrane of each of these consists of a ring of blue-staining endocuticle (Mallory's stain) separated from the exocuticle of the setal shaft by a ring of red-staining mesocuticle and connected to a similarly red-staining rim of mesocuticle.

The sense plates and sunken setae (both long and short) differ in that they stain red with Mallory's, black with Heidenhain's and pink with the Schiff polysaccharide test; as far as can be determined with the light microscope they are negative to osmic acid (see preceding section on sense plate epicuticle). These stain completely red with Mallory's, *i.e.*, unlike the sense hairs and sense pegs, they have no ring of blue-staining endocuticle.

In the pupa, the sense pegs become sclerotized first, sense hairs next, and sense plates and sunken setae last, but the time difference is not great and there is no reason to think it important since all reach whatever stage of sclerotization they are going to develop considerably prior to emergence of the adult. The real difference is that the hairs and pegs become completely sclerotized whereas the plates and sunken setae undergo only the first change (to mesocuticle). And since these changes occur early in relation to general sclerotization of the antennae, control must be somehow effected by the individual cells responsible for the formation of the various areas.

Incidentally, the tip of sunken setae may possibly be different from the shaft. At least in some slides, the tips of longer sunken setae show a minute spot of osmium deposit, or in Mallory's preparations, a corresponding blue spot. In some the tip even appears flared. Unfortunately the size of this spot at the tip of long sunken setae is close to the limits of vision with a light microscope.

The above notes comparing the various sensilla probably refer exclusively to the chitin-protein cuticle. Except for the sense plates (preceding section), details concerning the epicuticle could not be satisfactorily determined because of decreased visibility due to the cylindrical structure.

Comparison to Thoracic and Abdominal Cuticles

The preceding sections have dealt exclusively with the antennal cuticle. When this work was nearing completion, cursory examination was made to see if the novel features shown by antennae were peculiar to the antenna or just characteristic of honeybees. Only cuticles of teneral adult honeybees were examined.

The epicuticle of sclerites is negative to Heidenhain's haematoxylin. On the thorax it was negative to Mallory's stain and the Schiff polysaccharide test, but on the abdomen faint red staining was obtained on the tergites and stronger staining (especially with Mallory's) on the intersegmental membranes. It follows that

at least some areal differentiation of the epicuticle is found on other parts of the body besides the antennae but details remain to be worked out carefully. This is to be expected from the report of such areal differentiation in various species of insects (Richards, 1952b).

Most attention was given to the sclerotization picture in the chitin-protein subdivision. This clearly is not the same as on the antennae. On the thorax an outer exocuticle consists of a thin brown layer underlain by a broader amber zone; beneath this is a rather broad mesocuticle, and beneath this a thin endocuticle. On the abdominal tergum, endocuticle is limited to the intersegmental membranes but the sclerites consist of a more or less broad layer of amber exocuticle underlain by a somewhat thinner layer of mesocuticle. Adjacent to the intersegmental membrane are areas with microtrichia where the entire procuticle has been changed but only to mesocuticle.

Sclerites on the thorax and abdomen, then, present the orthodox differentiation picture. The pupal cuticle also shows the orthodox exo-, meso-, endocuticle sequence from outside to inside in sclerotized areas. These data show that the sclerotization sequence diagrammed in Figures 40–46 is a peculiarity of the antennae in honeybees, and on the antennae is shown by only the adult cuticle.

Summary

1. The epicuticle on honeybee antennae differentiates in correlation with sclerotization to give qualitatively different reactions on different parts.

2. The procuticle of the antennae shows a novel and unorthodox sequence of sclerotization involving two stages. The first stage begins at the outer surface and spreads inwardly, the second begins at the inner surface and spreads outwardly (Figs. 40–46). As a result the entire thickness of cuticle becomes sclerotized but still shows reducing power for osmic acid and ammoniacal silver nitrate. In contrast, the cuticle of thorax and abdomen shows the orthodox sequence of sclerotization proceeding from the outer surface inwardly.

3. Argentophile granules disappear during sclerotization from epidermal cells of areas that undergo sclerotization. Large numbers of argentaffin-positive granules remain in the cell cluster under the sense plates.

4. In the strip containing sense plates, the antennal cuticle develops a dark brown color in its inner half. This browning, which is additional to the usual sclerotization, is not found in the strip which is devoid of sense plates.

5. The cuticle over sense hairs and sense pegs becomes completely sclerotized; the cuticle over sense plates and sunken setae undergoes only the first of the two stages involved in sclerotization.

6. It follows that the cuticle over chemoreceptors cannot be assumed to have the same penetration properties as cuticle on other parts of the body. Also, different sensilla with cuticles of similar thickness cannot be assumed to have similar penetration properties.

7. The color of sclerotized antennal cuticle is not noticeably affected by prolonged treatment with hot water (100° C.) but lack of complete resistance to the treatment is shown by a change from near isotropic to readily recognizable birefringence.

8. The sense plates are differentiated early in the formation of the adult cuticle. They develop a complicated micelle pattern involving both radial and tangential orientations (Fig. 4). Other sensilla also differentiate early.

9. Balken are divisible into smaller fibrils of micellar dimensions. Viewed from the surface they form a crossed-fiber pattern on the antenna with four recognizable orientations: longitudinal, circumferential and two oblique (Fig. 1). At the ends of the segments these Balken fuse into rims which show completely circumferential orientation; the rims in turn join intersegmental membranes which show completely longitudinal orientation.

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